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Article

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Characterization of Extracts from the Bark of the Gabon Hazel Tree (*Coula edulis baill*) for Antioxidant, Antifungal and Anti-termite Products

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Abstract: Chemical composition of the bark extracts of *Coula edulis* was investigated to find potential antioxidant, anti-termite and antifungal compounds which can find useful applications in the fields of food, nutraceuticals, cosmetics or agrochemical. Phytochemical screening revealed the presence of several groups of active molecules such as alkaloids, polyphenols, flavonoids, saponins and sterols and/or terpenes in the different extracts. Total phenols, condensed tannins and flavonoids contents corroborated phytochemical screening. Gas chromatography-mass spectrometry (GC-MS) analysis revealed compounds in dichloromethane extract different from those obtained with all the other solvents. Hexadecanoic and trans-9-octadecenoic acids, as well as stigmasterol and β-sitosterol have been identified as the major compounds in the dichloromethane extract. Extracts obtained with acetone and toluene/ethanol mixture (2/1, v/v) indicated the presence of few amounts of fatty acids and sugars, catechin in small amount and huge amounts of phenolic acids like gallic and ellagic acids. The radical 2,2-diphenyl-1-picrylhydrazyle (DPPH) and the cationic radical 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) were used for evaluation of antioxidant properties of the different extracts. The dichloromethane extracts had a very low antioxidant activity, while acetone and toluene/ethanol extracts presented EC_{50} values similar to those of catechin and BHT used as reference antioxidant compounds. Effect of the different extracts of the bark of *C. edulis* on fungal growth inhibition indicated better inhibition of the mycelium growth of brown rot fungi compared to white rot fungi. Low anti-termite activities were recorded with the aqueous extracts, while stronger activities were recorded with dichloromethane, acetone and toluene/ethanol extracts.

Keywords: Antifungal; antioxidant; anti-termite; *Coula edulis baill*; extracts; valorisation

1 Introduction

Nature and its huge chemical diversity constitute an immense source of bio-inspiration for chemists. According to Newman et al. [1], nearly 52% of current drugs are made up or developed from natural products. Wood is an important natural resource containing numerous molecules of interest corresponding to secondary metabolites accumulated during the life of the trees. The Gabonese forest belonging to the forests of the Congo Basin is one of the largest forest in Africa after that of the Democratic Republic of Congo and Cameroon. According to the Gabonese Agency for Space Studies and Observations [2], it represents nearly 80% of the national territory with nearly 400 wood species, which may contain valuables biomolecules. Even if chemical composition of wood or bark extracts have been studied for some of these species, a majority of them have not been investigated up to now. This is the case of the hazel of Gabon (*Coula edulis*) considered by local populations as a multi-purpose species. Very durable and resistant to attack by fungi and insects, and more particularly those of termites, the wood of *C. edulis* is appreciated by the populations for its longevity [3]. Bark extracts are used in traditional pharmacopoeia like medicinal plant for the treatment of many diseases. In Ivory Coast, for example, bark decoction is used for purging or as an enema and for lumbar or kidney pain [4]. According to literature, bark is reported to contains high amounts of polyphenols among which tannin leading to many application [5,6]. Polyphenols are also reported to exhibit different biological activities, such as antifungal or antioxidant activities, which are responsible for their protective function in the tree. In addition to these activities numerous biological properties like antibacterial, antidiabetic or anticancer activities have been reported for bark extracts of different wood species [7–10]. In this context, it seemed interesting to investigate the chemical composition of *C. edulis* bark extracts for potential further applications. For this purposes, extractive contents, phytochemical screening, total phenols, tannins and flavonoids contents, GC-MS characterization and antioxidant, antifungal and anti-termite properties of these extracts were evaluated.

2 Materials and Methods

2.1 Reagents and Chemicals

All chemicals, reagents, malt extract for microbiology, agar and solvents were purchased from Sigma-Aldrich. Ultrapure water was produced by a PURELAB Option-Q equipment (Elga). Mayer's reagent (potassium mercuric iodide solution), iron perchloride 10% solution in water and Shinoda reagent (magnesium turn in hydrochloric acid) were prepared from reagent-grade chemicals according to well established procedures [11,12].

2.2 Fungi

Two types of basidiomycete fungi were used to carry out this work: Two white (or fibrous) rot fungi *Trametes versicolor* (TV) ((Linneus) L. Quélet strain CTB 863 A) and *Pycnoporus Sanguineus* (PS) and two brown (or cubic) rot fungi *Rhodonia Placenta* (RP) ((Fries) Cooke *sensu* J. Eriksson, strain FPRL 280), *Coniophora Puteana* (CP) ((Schumacher ex Fries) Karsten, strain BAM Ebw.15) have been used for growth inhibition tests.

2.3 Termite

Effect of extractives on termites was evaluated using *Reticulitermes flavipes* (ex.*santonensis de Feytaud*) collected from Soulac-sur-Mer (France). Termites are kept in breeding tanks in a dark, well-ventilated climatic enclosure set at a temperature of 27 ± 1°C and minimum relative humidity of 75%.

2.4 Bark Sampling

The bark was collected at breath height (1.3 m) from the log of a hazel Gabonese tree (*Coula edulis*) cut from a Gabonese primary forest in the area of Ayémé located in the province of Estuaire. Tree age was estimated to be between 50 and 75 years old. After air drying, the bark was ground into a fine powder using a ball mill (Retsch SM 100), sieved to a grain size (Ø = 0.160 mm) to optimize the extraction yields and then stored in glass jars after drying 24 hours in an oven at 70°C for future extractions.

2.5 Extraction

10 g of sawdust from the bark of *C. edulis* was successively extracted with solvents of increasing polarity, dichloromethane first followed by acetone, then toluene/ethanol mixture (2/1, v/v) followed by water. Two extraction methods using either Soxhlet or Dionex ASE 350 were investigated. With the Soxhlet method, extractions were performed for, 24 h before solvent evaporation under vacuum using a rotary vacuum evaporator, except for the aqueous extracts, which were lyophilized. For extraction with Dionex ASE 350, the same amount of sawdust was introduced into 60 mL cells. The extraction was carried out automatically during 15 minutes under to the combined effect of temperature and pressure (100°C and 100 bar). Once the extractions finished, the extracts were transferred into previously weighed flasks and the solvents removed using a rotary vacuum evaporator or lyophilized in the case of water. The extracts were then dried under vacuum in a desiccator in the presence of P_2O_5 and weighed regularly until constant mass. Each extraction was carried out in triplicate and the extractive content was determined using Eq. (1):

$$\textbf{Extractive content}\ (\%) = \frac{\mathbf{m}_{b+e} - \mathbf{m}_{b}}{\mathbf{m}_{s}} \times \mathbf{100} \tag{1}$$

where, $\mathbf{m_b}$ is the mass of the empty flask, $\mathbf{m_{b+e}}$ is the mass of the flask with the extractives after removal of the solvent and $\mathbf{m_s}$ the dry mass of sawdust before extraction.

2.6 Phytochemical Screening

Every needed reagent was prepared and used according to the protocols described in the literature [12,13]. All the different tests were carried out in triplicate.

Alkaloids were detected as follow: 20 mg of extracts and 10 mL of a dilute 10% sulfuric acid solution were poured into a test tube. The mixture was strong stirred for two minutes and some drops of Mayer's reagent were then added. The appearance of a yellowish precipitate was characteristic of the presence of alkaloids.

Flavonoids presence determination is obtained by dissolution of 2 mg of extracts in 2 mL of 95% ethanol with a few drops of hydrochloric acid and 0.5 g of magnesium ribbon, leading to a cherry pink color taken by the solution.

The detection of polyphenols was achieved by adding a drop of 10% aqueous iron perchloride solution in 2 mL of extracts solution(1 g/L) involving an intense blackish color appearance.

The saponins were identified by mixing 50 mg of extracts with 30 mL of distilled water in a water bath at 30°C for 5 minutes. After cooling, 10 mL of this solution were introduced into a test tube and vigorously vortex-shaken for 10 seconds. The presence of a 1 cm thick persistent foam indicates the presence of saponins.

Sterols and terpenes detection was carried out by mixing 20 mg of extracts, 2 drops of oleum, 10 drops of acetic anhydride into 3 mL of chloroform causing the appearance of a purple ring, turning blue and then green in the test tube.

2.7 Total Phenols Content

The determination of total phenols was carried out using the Folin-Ciocalteu colorimetric method [14] with a slight modification. First, a six point gallic acid calibration curve (0, 10, 30, 50, 80 and 100 ppm) was performed. The different extracts were dissolved in methanol. To carry out the assay, 0.5 mL of solution of extract dissolved in methanol and 2.5 mL of Folin-Ciocalteu reagent (diluted 10 times in distilled water) were successively introduced into a test tube. Then, 30 seconds to 8 minutes after adding the Folin-Ciocalteu reagent, 2 mL of sodium carbonate Na_2CO_3 (0.7 M) were added. A reaction blank containing no phenolic compounds is also produced. The reaction mixtures are stirred and incubated for 5 min at 50°C in a water bath. After this reaction time, all the samples were transferred to a cold water bath. the reaction mixtures are centrifuged (Eppendorf® Centrifuge 5702) at 4.4 rpm for 10 minutes. Then using a UV-Visible spectrophotometer (UV-2550, SHIMADZU) the absorbance is measured at 760 nm. The test was carried out in triplicate and the content of total phenols determined by means of the gallic acid calibration curve ($y = 0.0096x + 0.0058$; $r^2 = 0.9997$) by calculating the average concentration polyphenols present in extracts in mg equivalent of gallic acid/g of dry extract.

2.8 Condensed Tannins Content

The determination of condensed tannins or proanthocyanidins was carried out using the colorimetric method of acid condensation of vanillin [15] with a slight modification. First, a six point catechin calibration curve (0, 10, 30, 50, 80 and 100 ppm) was performed. The different extracts were then dissolved in methanol. To carry out the assay, a 0.5 mL solution of extract solution dissolved in methanol were mixed in a test tube with 3 mL of vanillin reagent also dissolved in methanol (4%, weight/volume). Then 1.5 mL of concentrated HCl (37%) were added and the mixture was kept in the dark at room temperature for 15 minutes. The absorbance of all the reaction mixtures corresponding to each point in the range and the samples is read at 500 nm using a UV-Visible spectrophotometer (UV-2550, SHIMADZU). The test was carried out in triplicate and the content of condensed tannin determined by means of the catechin calibration curve ($y = 0.0063x + 0.0759$; $r^2 = 0.9987$) by calculating the average concentration of flavonoids present in extracts in mg equivalent of gallic acid/g of dry extract.

2.9 Flavonoids Content

The flavonoid assay was carried out using the aluminum chloride colorimetric method described by [16] with a slight modification. First, a six points catechin calibration curve (0, 10, 30, 50, 80 and 100 ppm) was performed. The different extracts were then diluted in methanol. To carry out the assay, 0.5 mL of the extract solution diluted in methanol, 2 mL of distilled water and 1 mL of aluminum chloride (10%) are successively introduced into a test tube, and leaved for 6 minutes. Then, 1 mL of 1 M potassium acetate is added. After incubation at room temperature for 30 minutes, the absorbance of all the reaction mixtures corresponding to each concentration in the calibration curve and the samples were measured at 415 nm using a UV-Visible spectrophotometer (UV-2550, SHIMADZU). The test was carried out in triplicate and the flavonoid content determined using the catechin calibration curve ($y = 0.0088x + 0.0818$; $r^2 = 0.9989$) by calculating the average concentration of flavonoids present in extracts in mg equivalent of gallic acid/g of dry extract.

2.10 GC-MS Analysis

The GC-MS analysis was carried out with a 95% dimethyl/5% diphenylpolysiloxane column (30 m × 0.25 mm × 0.25 µm) on a Perkin Elmer Clarus 680 chromatograph coupled to a SQ8 mass spectrometer, controlled by Turbomass v6.1 software and having the 2011 edition NIST database. The samples were derivatized to facilitate the detection of all the present compounds by pouring 50 µL of N,O-bis (trimethylsilyl) trifluoroacetamide containing 1% of trimethylchlorosilane on 1 to 2 mg of dry extracts in a 2 mL vial. This preparation was incubated for 2 hours at 70°C, closed vial in order to perform the

silylation reaction. The vial was then opened to allow BSTFA evaporation and the remaining silylated extracts were dissolved in 1 mL of ethyl acetate. 1 µL of this solution was injected in splitless mode into the gas chromatograph at an inlet temperature of 250°C. A typical oven program was: 80°C for 2 minutes, heating to 10°C min^{-1} to 190°C, followed by 15°C min^{-1} to 280°C and maintained at this temperature for 10 minutes, heated again at 10°C min^{-1} at 300°C and maintained at this temperature for 14 minutes. If necessary, the heating program was slightly modified to improve the resolution of certain samples, leading to different retention times for one compound. A mobile phase of 1 mL min^{-1} of helium was used. After the separation step, the compounds were transferred by a transfer line heated to 250°C to the mass spectrometer and ionized by electronic impact with an ionization energy of 70 eV. The compounds were then identified by comparison of their mass spectrum with the NIST library via NIST MS Search 2.0 (2011). The identification was considered relevant for matching and reverse matching when the values of the coefficients were greater than 900.

2.11 Antioxidant Activity

Antioxidant activity of the different extracts was evaluated using (2,2-diphenyl-1-picrylhydrazyl) radical (DPPH) or 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic) cationic radical ($ABTS^{+}$). Catechin and butylated hydroxytoluene (BHT) were used as reference antioxidant compounds.

2.11.1 DPPH Free Radical Method

The antioxidant activity was estimated using the radical (2,2-diphenyl-1-picrylhydrazyl) or DPPH inspired by the method described by Brand-William et al. [17]. DPPH was prepared by mixing 39.4 mg of solid DPPH in 100 mL of pure methanol. Then 20 mL of this solution was taken and diluted in a 100 mL flask. Extracts at different concentrations were dissolved in methanol. Then, 1 mL of DPPH and 1 mL of extract dissolved in methanol were introduced in a test tank and the mixture stored in the dark at room temperature for 30 minutes. After this incubation time, the absorbance is measured at 517 nm using a UV-Visible spectrophotometer (UV-2550, SHIMADZU). The test was carried out in triplicate and the antioxidant activity of extracts (AA) was calculated in percentage relative to the control (DPPH alone in methanol without extract) according to the following Eq. (2):

$$\mathbf{AA}\ (\%) = \frac{\mathbf{A_0} - \mathbf{A}}{\mathbf{A_0}} \times \mathbf{100} \tag{2}$$

where $\mathbf{A_0}$ is the absorbance of the control (DPPH alone in methanol without extract) and **A** the absorbance of the DPPH solution in the presence of extracts. The effective concentration for consuming 50% DPPH (EC_{50}) is determined from the equation of the curve representing the percentage of inhibition as a function of the concentrations of the samples. The low EC_{50} concentrations correspond to high antioxidant activities.

2.11.2 $ABTS^{+}$ Free Cationic Radical Method

The antioxidant activity was also estimated using $ABTS^{+}$ inspired by the protocol of Simona et al. [18] with a slight modification. $ABTS^{+}$ (7 mM) was prepared by mixing 384 mg of solid ABTS in 100 mL of potassium persulfate (2.45 mM). The solution was then diluted in a sodium phosphate buffer solution (5 mM) of pH = 7 in order to have an absorbance at 1.4 at 734 nm. Extracts at different concentrations are dissolved in methanol. In test tubes, 1 mL of $ABTS^{+}$ and 1 mL of extract dissolved in methanol are introduced. The whole is stored in the dark at room temperature for 30 minutes. After this incubation time, the absorbance is recorded at 734 nm using a UV-Visible spectrophotometer (UV-2550, SHIMADZU). The test is carried out in triplicate and the antioxidant activity (AA) for each concentration was calculated according to Eq. (2). Where $\mathbf{A_0}$ is the absorbance of the control ($ABTS^{+}$ alone in methanol without extract) and **A** the absorbance of the solution of $ABTS^{+}$ in the presence of extracts.

The effective concentration for consuming 50% of $ABTS^{+}$ (EC_{50}) is determined as previously from the curve representing the percentage inhibition as a function of the concentrations of the samples.

2.12 Fungal Growth Inhibition Tests

Effect of extracts on fungal growth inhibition was determinate according to the method described by Chang et al. [19] with a slight modification. The different extracts were dissolved in a minimum of ethanol (1 mL maximum). The agar media were prepared in 100 mL Erlenmeyer flasks by adding 4 g of extracted malt, 2 g of agar and 96 g of water. The agar solutions are sterilized in an autoclave at 120°C for 25 minutes. Then, 50 mg or 100 mg of the different extracts dissolved in the minimum amount of ethanol (1 mL maximum) were added to the sterilized agar media, so that to obtain a final extract concentration of 500 ppm or 1000 ppm, respectively. The mixtures were prepared near a flame in a laminar flow hood in sterile condition, then homogenized and left to stand. After cooling (to around 40°C), each Erlenmeyer flask containing the agar media at different extract concentrations was well homogenized and about 20 mL of sterilized agar media were distributed in Petri dishes under sterile conditions. After solidification, the media were inoculated with a freshly cultivated mycelium disc of the various fungal strains. For each fungus and each concentration, three Petri dishes with extract and three Petri dishes with ethanol only as a control were prepared. The dishes were incubated at 22°C and 70% Relative Humidity (RH) in a Memmert climate chamber. The growth of the fungi was measured daily, until the control Petri dishes were completely covered with mycelium (10 to 12 days). The tests were then stopped and the evaluation of the fungal growth are presented in the form of growth curves as a function of incubation time.

2.13 Anti-Termite Activity

70 µL of extract solution (500 ppm and 1000 ppm in ethanol) were impregnated on Whatman filter papers previously conditioned at 20°C, 65% RH were weighed ($\mathbf{m_0}$) before being exposed to termites (*Reticulitermes flavipes*). The papers impregnated with the various solutions were air dried (20°C–65% RH for 2 hours) and weighed ($\mathbf{m_1}$). The tests were carried out in 9 cm Petri dishes, in which 40 g of wet Fontainebleau sand (1 volume of water for 4 volumes of sand) were placed on the periphery. The impregnated Whatman papers were placed on a plastic grid (to avoid moisture saturation) in the middle of the Petri dish and 20 termite workers were added to each Petri dish. The Petri dishes were then stored in the dark at 27°C, 75% RH. Papersoaked in ethanol or water only were also tested as controls and Petri disheswithout filter paper were used as diet control. Petri dishes were monitored regularly and the test stopped when all the worker termites in the diet control Petri dishes died (21 days approximately). At the end of the tests, the alive termites are counted and the survival rate of the termites was determined as indicated in Eq. (3). The samples were cleaned and dried in the open air then weighed ($\mathbf{m_2}$) to determine the mass losses due to termite attacks (ML_{ter}) according to Eq. (4)

$$\textbf{Survival rate}\ (\%) = \frac{\textbf{Number of worker termites alive after the test}}{\textbf{Number of worker termites used for the test}} \times \mathbf{100} \tag{3}$$

$$\mathbf{ML_{ter}}\ (\%) = \frac{\mathbf{m_1 - m_2}}{\mathbf{m_1}} \times \mathbf{100} \tag{4}$$

where $\mathbf{m_0}$ was the initial stabilized mass of Whatman paper before impregnation, $\mathbf{m_1}$ the mass of Whatman paper after impregnation and air drying before exposure to termites, $\mathbf{m_2}$ the mass of Whatman papers after impregnation and air drying after exposure to termites.

2.14 Statistical Analysis

XLSTAT 2019 was used and the results were known as the main values representing the mean of the repetitions ± the standard deviations. The Spearman correlation test was carried out between the EC_{50}, the total content of phenols, condensed tannins and flavonoids. The values are statistically significant at $\rho < 0.05$.

3 Results and Discussion

3.1 Extractives Contents

Extractives content (Tab. 1) depends of the solvent used and to a lesser extent from the method used for extraction. Amounts of extracts are, like very often for tropical wood species described to content high amounts of extractives, in agreement with bibliographic data [20–22]. The extractives contents obtained with dichloromethane are practically similar independently of the extraction method used indicating non negligible amounts of apolar compounds. The highest extractives contents were obtained with acetone and water indicating probably the presence of the high amounts of polar compounds like phenolic compounds and sugar. Results obtained with toluene/ethanol mixture indicated different amounts of extractives depending of the method used, Soxhlet extraction leading to higher yields of extracts, which may result from ethanolysis during extraction. Indeed, shorter extraction time used during solvent pressurised extraction with Dionex are reported to limit by-products formation. The total bark extractives contents obtained with Soxlhet (21%) are higher than those obtained with Dionex (17.8%). Numerous studies carried out on several tropical species reported quite similar high amounts of bark extractives [23–28].

Table 1: Extractive content in *C. edulis* bark sawdust

Solvant	Extractive content (%)[a]	
	Soxlhet	Dionex ASE 350
Dichloromethane	4.1 ± 1	4.1 ± 0.2
Acetone	7 ± 0.7	6.8 ± 0.8
Toluene/ethanol	3.6 ± 0.3	0.6 ± 0.2
Water	6.3 ± 0.1	6.3 ± 1.2
Total	**21**	**17.8**

[a]Values represent means of three replicates ± standard deviation.

3.2 Phytochemical Screening

Sterols and terpenes were detected in dichloromethane extracts and acetone. Such groups of compounds have been described in the literature to possess antifungal and anti-termite properties [25,29–37]. Alkaloids, polyphenols, flavonoids and saponins were detected in all extracts except those obtained with dichloromethane. Such groups are known in the literature as potential antioxidant, antibacterial, antifungal, antimicrobial and termicidal compounds [22,38–41]. Phytochemical screening is reported in Tab. 2.

Table 2: Phytochemical analysis of the main groups in *C. edulis* bark

Active compounds	Test procedure	Extraction solvent[a]			
		Dichloromethane	Acetone	Toluene/Ethanol	Water
Alkaloids	Mayer's reagent	−	+	+	+
Flavonoids	Shinoda test	−	+	+	+
Polyphenols	Iron (III) chloride	−	+	+	+
Saponins	Aphrogenic power	−	+	+	+
Sterols and terpenes	Liebermann-Bouchard	+	+	−	−

+/− = presence/absence of the groups. [a]solvent used for extraction.

3.3 Phenolic Compounds

Total phenols, condensed tannins and flavonoids contents vary from one solvent to another (Tab. 3). The total phenol content varies between 733.1 and 552.8 mg EqGA per gram of extract, while condensed tannins content varies between 192.2 and 122.4 mg EqC per gram of extract and flavonoids content between 60.2 and 30.1 mg EqC per gram of extract. Independently of the test performed, the highest contents of phenolic compounds were obtained with the acetone extracts and the lowest with the aqueous extracts. This is easily explained by the polarity of the solvent used during our successive extractions, the higher polarity of water allowing to extract higher polarity compounds such sugar, while acetone is known to extract mainly phenolic compounds [42]. These contents in total phenols. condensed tannin and flavonoids are similar to those found by Saha Tchinda et al. [20] during their study on antioxidant activity, total phenol content and chemical composition of extracts from four Cameroonian woods: Padouk (*Pterocarpus soyauxii Taubb*). tali (*Erythrophleum suaveolens*). moabi (*Baillonella toxisperma*) and movingui (*Distemonanthus benthamianus*).

Table 3: Total phenols, condensed tannins and flavonoids contents in the different extracts of *C. edulis* bark

	Extraction solvent			
	Dichloromethane	Acetone	Toluene/Ethanol	Water
Total phenols (mg EqGA g^{-1})[a]	–	733.1 ± 1.3[c]	552.8 ± 1.7[c]	561.5 ± 1.3[c]
Condensed tannin (mg EqC g^{-1})[b]	–	192.2 ± 1.5[c]	156.6 ± 1.3[c]	122.4 ± 1.1[c]
Flavonoid (mg EqC g^{-1})[b]	–	60.2 ± 0.9[c]	58.6 ± 1.3[c]	30.1 ± 1.1[c]

[a]mg EqGA g^{-1}: Milligram equivalent of gallic acid per gram of dry extract.
[b]mg EqC g^{-1}: Milligram equivalent of catechin per gram of dry extract.
[c]Mean value of three replicates ± standard deviation.

3.4 GC-MS Analysis

Chromatograms obtained with the different extracts excepted water are presented in Fig. 1.

Identification of the chemicals present in the different extracts using the NIST database is presented in Tab. 4.

The results obtained from the GC-MS analysis of the different extracts of the bark of *C. edulis* are in agreement with those of phytochemical screening and those obtained during the assays of phenolic compounds. Indeed, the analysis of dichloromethane extracts indicated the presence of fatty acids and triterpenes like stigmasterol, β-Sitosterol and lupeol, which may react with the Liebermann-Bouchard test, but can also be part of saponins structures involving hydrophobic terpenoid moiety and hydrophilic sugar moiety. Different phenolic compounds like gallic and ellagic acids or catechin have been identified in acetone and toluene/ethanol extracts corroborating the different tests indicating the presence of phenolic compounds. The presence of high amounts of gallic and ellagic acids, as well as numerous sugar compounds, in the last extraction using toluene/ethanol reflects probably the presence of hydrolysable tannins whose ethanolysis or hydrolysis leads to a significant presence of these compounds, even after prior extraction with acetone. Such phenomena may also explain the presence of fatty acid from the hydrolysis of monoglyceride or suberin.

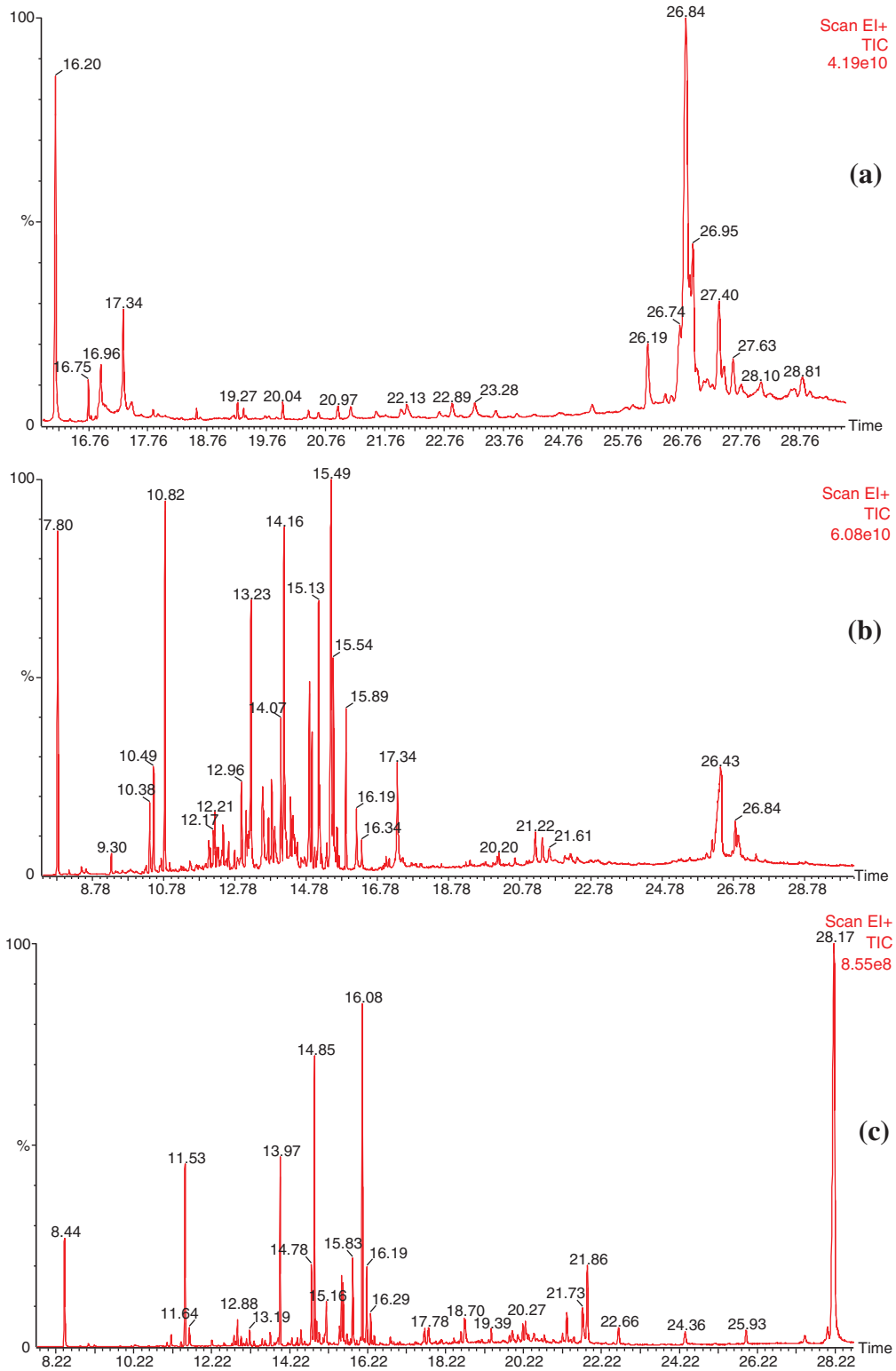

Figure 1: Chromatograms of the different extracts from the bark of *C. edulis*. a) dichloromethane extract. b) acetone extract. c) toluene/ethanol extract

Table 4: Compounds identified by GC-MS and their abundance relative to TIC[a] in extracts from the bark of *C. edulis*

Solvent	Retention time (min)	Compound	Abundance (%)
Dichloromethane	16.20	hexadecenoic acid	8
	17.34	trans-9-Octadecenoic acid	3
	22.13	1-Monolinoleoylglycerol	1
	26.19	Stigmasterol	7
	26.84	β-Sitosterol	42
	27.40	Non identified	14
	27.63	Lupeol	7
	28.81	Non identified	16
Acetone	7.80	Glycerol	8
	10.49/10.82	meso-Erythritol	2/9
	12.17	D-(-)-Ribofuranose	1
	12.21/12.96	β-L-Mannopyranose	1/2
	13.23	Xylitol	9
	14.07	D-(-)-Fructofuranose	3
	14.16	d-(-)-Fructose	9
	15.13	D-Sorbitol	5
	15.49	Gallic acid	12
	15.54	β-D-Allopyranose	4
	15.89	Inositol	3
	16.19	hexadecenoic acid	2
	16.34	Myo-Inositol	1
	17.34	trans-9-Octadecenoic acid	6
	21.22	Catechin	1
	26.43	Ellagic acid	14
	26.84	Non identified	6
Toluene/Ethanol (2/1, v/v)	8.44	Glycerol	4
	11.53	meso-Erythritol	6
	13.97	Arabinitol	6
	14.78/14.85	D-(-)-Fructofuranose	3/8
	15.83	Mannitol	3
	16.08	Gallic acid	9
	16.19	D-Glucose	3
	20.27	Non identified	5
	21.73	Octadecanoic acid	3
	21.86	Stearic acid	4
	28.17	Ellagic acid	43

[a]TIC = Total Ion Current.

3.5 Antioxidant Activity

The antioxidant activity of the different bark extracts using DPPH or $ABTS^+$ methods were evaluated by measuring their EC_{50} values, corresponding to the concentration of extract necessary for to trap 50% of the free radical used (Tab. 5). The lowest EC_{50} value is, the higher the antioxidant activity is. The ability of the extracts of the bark of *C. edulis* to trap free radicals varies depending on the extraction solvent. Dichloromethane extracts present very low trapping capacity of free radicals as evidenced by the high values of EC_{50} recorded, 795.3 ppm with DPPH and 532.8 ppm with $ABTS^+$. In contrast, all the other extracts have shown an interesting capacity to trap free radicals. The highest activities were recorded with the acetone extracts followed by toluene/ethanol extracts, whose EC_{50} values approximate the values of the antioxidant activities of catechin and BHT used as representative antioxidants used in industry. Water extracts presented slightly lower antioxidant activities compared to activities of acetone and toluene/ethanol extracts, but much better than that of dichloromethane extract. These results matched perfectly with chemicals analysis and phytochemical screening indicating the presence of phenolic compounds in acetone, toluene/ethanol and water extracts. Similar studies already described in the literature correlated antioxidant properties of extracts to their phenolic compounds contents [43–45].

Table 5: Antioxidant activity from the different extracts from the bark of *C. edulis*

	Bark of *C. edulis*		Catechin		BHT	
	DPPH EC_{50}[a]	$ABTS^+$ EC_{50}[a]	DPPH EC_{50}[a]	$ABTS^+$ EC_{50}[a]	DPPH EC_{50}[a]	$ABTS^+$ EC_{50}[a]
Dichloromethane	795.3 ± 0.4	532.8 ± 0.2	3.0 ± 0.2	1.05 ± 0.4	4.1 ± 0.2	2.08 ± 0.2
Acetone	9.6 ± 0.2	5.6 ± 0.5				
Toluene/ethanol	10.1 ± 0.4	7.6 ± 0.2				
Water	21.1 ± 0.6	10.2 ± 0.7				

DPPH: 2.2-diphenyl-1-picrylhydrazyl. $ABTS^+$: 2.2-azinobis (3-éthylbenzothiazoline-6-sulfonic acid). EC_{50}: Concentration in ppm required to remove 50% of radicals. [a]Values represent means of three replicates ± standard deviation.

3.6 Antifungal Activity

In all cases, extracts present a retarding effect on the growth of the different fungal strain tested, which was more pronounced in the case of brown rot fungi than white rot (Fig. 2). At 1000 ppm, all extracts were able to inhibit totally the growth of brown rot fungi *R. placenta* and *C. puteana*, while only partial inhibition of the fungal development was observed for white rot fungi *P. sanguineus* and *T. versicolor*. At 500 ppm, all extracts lead only to a partial inhibition of the fungal growth independently of the fungal strain tested. In all cases, effect of extractives present in the different extracts seems more fungistatic than fungicidal. Difference in behaviour of the extracts between white rot and brown rot fungi may be due to their difference of mechanisms involved in wood polymer degradation. White rot secretes oxidative enzymes such as laccases and peroxidases, which may be able to degrade phenolic compounds by oxidation. These enzymes are capable of metabolizing phenolic compounds limiting their toxicity and therefore the effectiveness of the extracts to inhibit the growth of the mycelium [46]. Brown rot were, on the contrary, less effective to degrade phenolic compounds, resulting in a lower ability to detoxify the medium. These results are in agreement with those obtained described in the literature [47–50,28,22]. Phenolic compounds are also known to affect the membranes of microorganisms causing leakage of the cytoplasmic content [51]. In this last case, the mechanism of inhibition depends on the ability of phenolic functions to affect the membranes of cellular lipoproteins, causing the weakening of ionic cell homeostasis which destroys the entire cellular structure [52]. Analysis of the dichloromethane extract of

the bark have highlighted the presence of triterpenes such as stigmasterol or β-Sitosterol known in the literature to present antifungal properties [30,33,37], which can be at the origin of the activity of this extract.

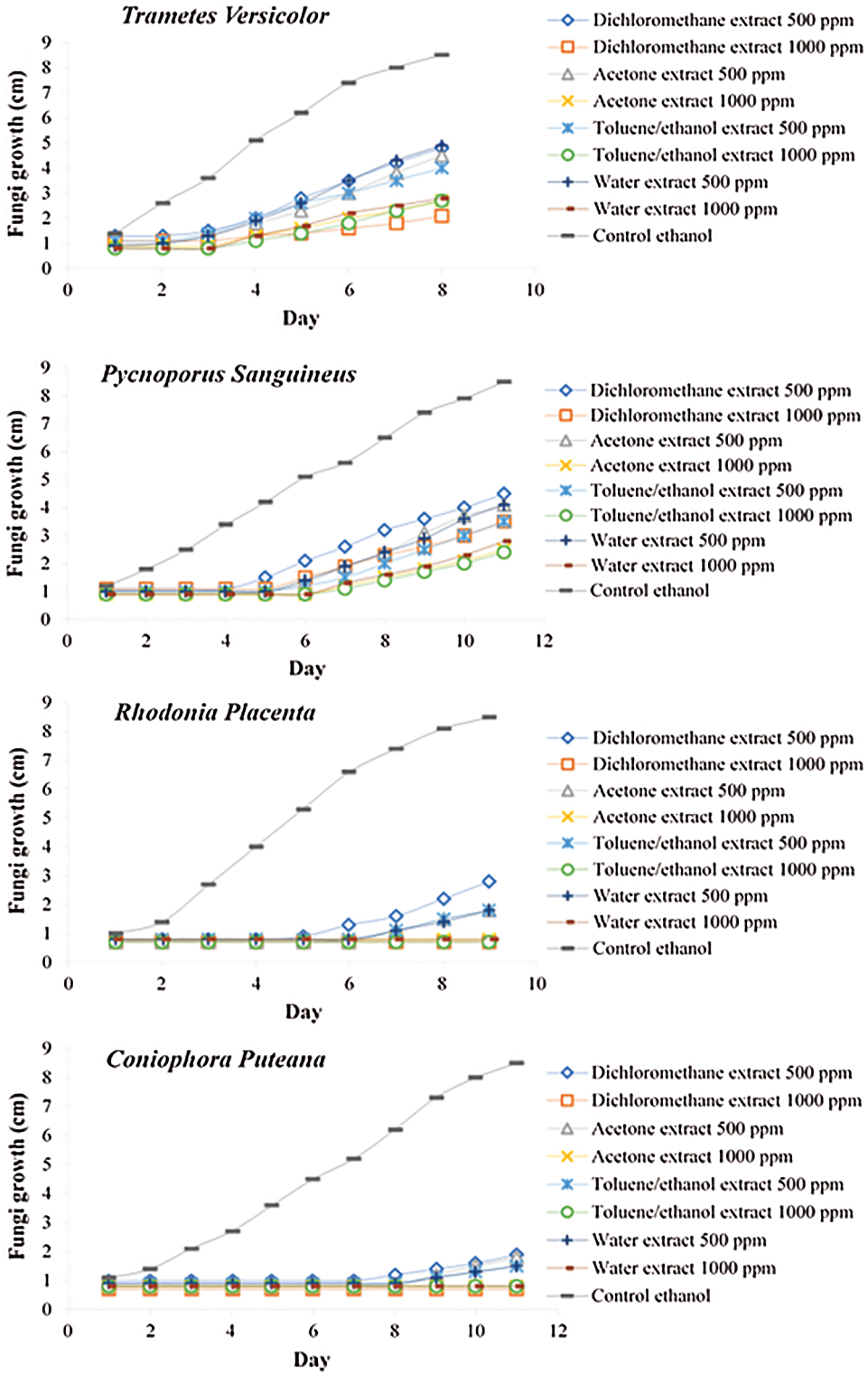

Figure 2: Growth inhibition effect of the extracts on different fungi

3.7 Anti-termite Activity

Water extracts of the bark of *C. edulis* presented low anti-termite activities with termite's survival rates of 80 and 71.7% and mass losses of 41.6 and 37.3% at 500 ppm and 1000 ppm, respectively (Tab. 6). Dichloromethane, acetone and toluene/ethanol presented more interesting anti-termite activities with survival rates of 30, 58.3 and 65% and mass losses of 14.6, 22.1 and 28.9% at 500 ppm and survival rates of 1.7, 6.6 and 8.3% and mass losses of 8.6, 6.7 and 8.3% at 1000 ppm. Stigmasterol and β-sitosterol present in the dichloromethane extract could explain this activity. Indeed, according to literature, terpenes and terpenoids have been reported to present toxic, antifeeding and repellent properties against termites and other insects [29,31–32,34–35,37]. For the acetone extracts and with the toluene/ethanol mixture. The phytochemical screening and the GC-MS analysis made it possible to demonstrate the presence of phenolic compounds such as gallic acid and ellagic acid which are generally different fragments of the structure of the tannins and which could explain the good anti-termite activity of these extracts vis-à-vis the termites. These results are corroborated by the literature which explains that tannins, due to their protein binding properties and the inhibition of several digestive enzymes, are known to be strongly astringent [53].

Table 6: Anti-termite activity tested on Whatman paper filters impregnated with different concentrations of *C. edulis* bark extracts

Extract	Concentration	Survival rate (%) [a]	ML_{ter} (%) [a]
Dichloromethane	500 ppm	30.0 ± 30.0	14.6 ± 9.3
	1000 ppm	1.7 ± 2.9	8.6 ± 6.0
Acetone	500 ppm	58.3 ± 11.6	22.1 ± 8.1
	1000 ppm	6.6 ± 7.6	6.7 ± 1.6
Toluene/Ethanol	500 ppm	65.0 ± 10.0	28.9 ± 4.8
	1000 ppm	8.3 ± 7.6	8.3 ± 1.8
Water	500 ppm	80.0 ± 2.9	41.6 ± 2.7
	1000 ppm	71.7 ± 15.3	37.3 ± 8.7
Wathman paper		70 ± 16.1	37.3 ± 6.8
Wathman paper + ethanol		78.3 ± 15.9	28.3 ± 4.3
Diet control (no paper)		0 ± 0.0	0 ± 0.0

[a]Mean value of three replicates ± standard deviation.

4 Conclusion

Chemical composition of *C. edulis* bark was investigated and elucidated for the first time. Phytochemical screening indicated the presence of terpenes and sterols in dichloromethane and acetone extracts, while alkaloids, flavonoids, polyphenols and saponins were detected in acetone, toluene/ethanol and water extracts. Determination of total phenols, condensed tannins and flavonoids contents corroborated results issued from phytochemical analysis. Further investigations carried out using GC-MS analysis confirmed the presence of terpenes in dichloromethane extracts with stigmasterol, lupeol and β-sitosterol as the major compounds and fatty acids. Characterization of more polar fractions indicated the presence of gallic and ellagic acid, catechine and different sugars. High amounts of gallic and ellagic acids in the toluene/ethanol extracts suggested the presence of hydrolysable tannins, which may be degraded during extraction leading to phenolic acid. Characterization of antioxidant properties of acetone

and toluene/ethanol extracts indicated quite similar antioxidant activities than BHT or catechin used as antioxidant reference compounds. Aqueous extract presented also interesting antioxidant value, while dichloromethane extract presented no activity. Growth inhibition tests performed on different brown and white rot fungi indicated fungistatic effects resulting in a delay of the mycelium growth, brown rots being more susceptible than white rots. Anti-termite activity measured after impregnation of the different extracts on Whatman filter paper revealed weak activities for aqueous extracts, while other extracts presented more interesting anti-termite, efficacy increasing with the solution concentration. Extracts obtained from the bark of *C. edulis* could therefore be interesting compounds for the formulation of bio-pesticides with potential lower effect on the environment due to their natural origin. Moreover, antioxidant properties may be of valuable interest for food and cosmetic industries and other sector, where such properties are required.

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